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COMPARISON OF PHENOLICS, FLAVONOIDS AND ANTHOCYANINS IN PIGMENTED AND NON-PIGMENTED MAIZE IN BANGLADESH

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Abstract

In the present study the composition of phenolics, flavonoids and anthocyanins in a purple maize (SAU Bhutta 3) variety were determined and compared its phytonutrients with those of other maize varieties cultivated in Bangladesh. The SAU purple maize contained 106.0 ± 8.3 and 165.1 ± 8.0 mg /100 g of methanol soluble and acidic methanol soluble polyphenols, respectively. The SAU Bhutta 3 had the greatest amount of flavonoids (77.0 ± 10.0 mg /100 g) and anthocyanins (69.0 ± 5.0 C3G equv./100 g). Nevertheless, the SAU white maize variety had 31.0 ± 2.0, 47.0 ± 4.4 mg FAE/100 g and 9.0 ± 2.0 mg CE/100 g of free and bound phenolics and total flavonoids, respectively, which were lower than those of the SAU Bhutta 3. Again, the SAU red maize contained the lowest level of total anthocyanins (4.11 ± 1.2 mg C3G/100 g). The SAU Bhutta 3 exhibited higher proportions of insoluble phenolics compared to total phenolics and flavonoids compared to insoluble phenolics. Overall, the SAU Bhutta 3 contained relatively high amounts of phenolics, flavonoids and anthocyanins; thus, this particular maize variety shows significant potential for future utilization.

Introduction

Maize kernels are the largest seed-storage food among cereals. Maize kernels possess both pigmented and non-pigmented pericarps, with pigmentation attributed to a variety of colored phytochemical compounds and secondary metabolites. The primary pigments or compounds found in maize pericarp include various types of phenolics, flavonoids, anthocyanins, carotenoids, and more. Notably, among these color compounds, phenolics are particularly abundant in maize (Adom and Liu 2002, Pandey and Rizvi2009). The primary categories of polyphenols could encompass phenolic acids, flavonoids, anthocyanins, stilbenes, and lignans (Rigacci 2015). Flavonoids exhibit a broad spectrum of colors ranging from light yellow to blue, and they play a key role in determining the color of the pericarp (Santos *et al.* 2017). The major types of flavonoids identified in cereal grains are flavonols, anthocyanins and proanthocyanidins. Anthocyanins are water-soluble phenolics that result in red, purple and blue coloration in cereal grains (Bueno *et al.* 2012). Numerous research findings indicate that incorporating phenolic-rich diets into one's meal plan offers significant health advantages. These diets have been associated with anti-cancer, heart-protective, anti-inflammatory, neuroprotective, cholesterol-lowering, blood sugar-regulating, and lipid-lowering effects. Additionally, they have demonstrated anti-tumor properties and the ability to decrease oxidative stress (Adom and Liu 2002, Wu *et al.* 2016, Leuci *et al.* 2020,). The color of maize also has many industrial uses, for example, for preparing drinks and producing edible colors (Nuss and Tanumihardjo 2010, Colombo *et al.* 2021).

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In Bangladesh, maize primarily serves as flour, grit, and semolina in household settings. Roasted corn on cobs is popular as a street food, while corn starch and syrup find applications in the food, paper, and pharmaceutical industries. Nonetheless, there has been a noticeable rise in the annual demand for maize, with a corresponding increase in maize production observed in recent years (2022-23) (Ali *et al.* 2010, BBS 2023, CYMMIT 2023). To meet the demand, several maize varieties have been released by the government, organizations outside government control, educational institutions, and agricultural seed companies in Bangladesh (BARI 2022-2023, mofood 2023). In recent years, at Sher-e-Bangla Agricultural University, Dhaka has released a purple maize named ‘SAU Bhutta 3’, which has a purple pericarp, to meet the continuously increasing demand for maize. However, Yellow or white maize is commonly grown and used for various purposes in Bangladesh. Additionally, there are indigenous cultivars, both pigmented and non-pigmented, found primarily in the hilly regions, which are also utilized in Bangladesh for consumption as food by the indigenous people. Estimation of phenolic compounds in such pigmented maize cultivars is necessary to explore their nutritional potential for health benefits as food as well as for industrial purposes. Hence, the aims of this study were to ascertain the levels of polyphenols (methanol and acidified methanol-soluble), flavonoid, and anthocyanin contents in both pigmented and non-pigmented whole-grain maize varieties cultivated in Bangladesh.

Materials and Methods

Three different maize (white, red, and SAU Bhutta 3) cultivars were kind donation from the plant breeding department of Sher-e-Bangla Agricultural University in Dhaka. Pericarp colors of these maize kernels were identified as white, red, and purple, respectively. Notably, the endosperm of all kernels from these varieties was white. Additionally, a yellow maize variety, BARI hybrid maize-9, with yellow seed coat and endosperm, was obtained from the Bangladesh Agricultural Research Institute (BARI). Other samples include deep red maize and multicolored maize were local varieties sourced from the southeastern hilly regions of Bangladesh. The kernel pericarp color of the deep-red maize kernels ranged from deep red to blackish, while the kernel color of the multicolored maize kernels varied from yellow to deep red. Both samples had white endosperms (Table 1 and Fig. 1). Maize kernels, which had been sun-dried and cleaned, were ground into flour using a grinder (Miyako, model no: YT-4677A-S). The resulting maize flours were stored in airtight containers and kept refrigerated at −20°C.

The methanolic and acidified methanol-soluble phenolics extracts of the samples were prepared following the method described by Hossain and Jayadeep (2022). The content of methanol-soluble polyphenols and acidified methanol-soluble phenolics in maize was determined by the Folin-Ciocalteu reagent method (Singleton *et al.* 1999). Flavonoids was extracted from defatted samples by methanol solvent (acidified with 1% HCl). The extract was utilized for estimating the total flavonoid content following the methods by Hossain and Jayadeep (2022). Anthocyanin content was determined from varying volumes of acidified methanolic maize extract at two distinct wavelengths (515 and 700 nm). The content of anthocyanin was estimated from the absorbance (Urias-Lugo *et al.* 2015).

The data were computed based on dry weight are expressed as the mean ± standard deviation (SD) of 3 assessments for each parameter. Statistical comparisons of the sample components' means were conducted using one-way ANOVA and Tukey’s test. The confidence level was set at 95%. The IBM SPSS 20 statistical software (IBM Corp., Armonk, NY, USA) was used for analysis.

Table 1. Different maize cultivars used in the study.

Sl. no.	Maize cultivars	Source*	Pericarp color
1.	SAU white maize	SAU	white
2.	SAU red maize	SAU	red
3.	SAU purple maize (SAU Bhutta 3)	SAU	purple
4.	BARI hybrid maize 9	BARI	yellow
5.	Deep red maize	Bandarban district	red to blackish
6.	Multicolored maize	Bandarban district	Yellow to deep red

*SAU: Sher-e-Bangla Agricultural University, BARI: Bangladesh Agricultural Research Institute, Bandarban district is a hilly region of Bangladesh.

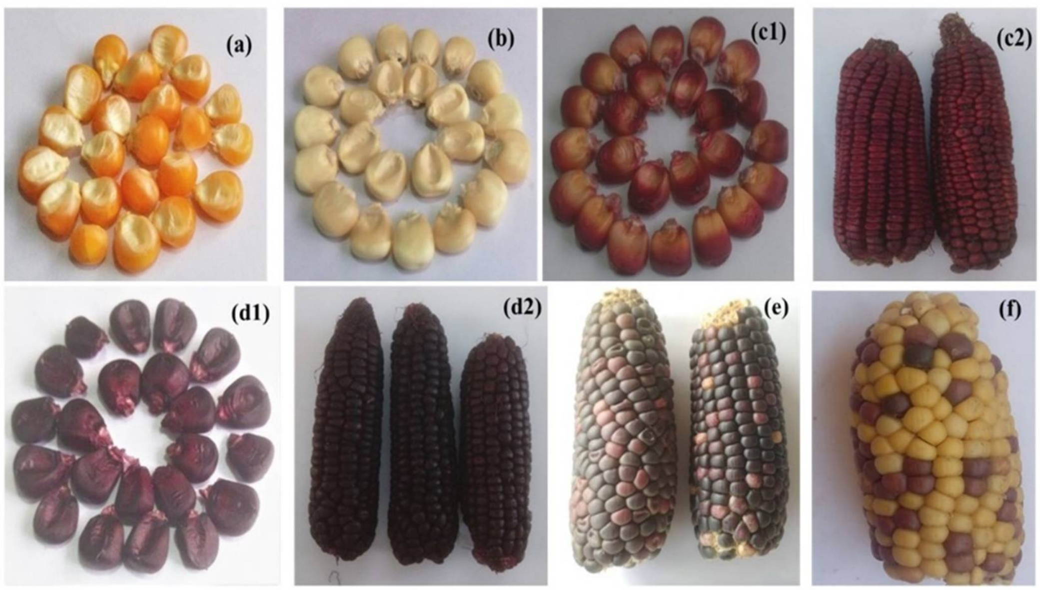

Fig. 1. Experimental samples: (a) BARI hybrid maize 9, (b) SAU white maize, (c) SAU red maize (kernel c1, cob c2), (d) SAU purple maize (kernel d1, cob d2), (e) Deep red maize and (f) multicolored maize.

Results and Discussion

The methanol soluble phenolics content in various maize varieties varied from 31.0 ± 2.0 to 106.0 ± 8.3 mg FAE/100 g sample (Fig. 2). Among these, SAU Bhutta 3 and deep red maize exhibited the highest methanol soluble phenolic contents, measuring 106.0 ± 8.3 and 105.0 ± 9.0 mg FAE/100 g sample, respectively. The SAU white maize variety exhibited the lowest quantity (31.0 ± 2.0 mg FAE/100 g sample) of methanol soluble phenolics. Conversely, the BARI hybrid maize 9, SAU red maize, and multicolored maize variants demonstrated comparable levels of methanol soluble phenolic content. The relatively lower concentration of methanol soluble phenolics observed in this study aligns with previous findings (Lopez- Martinez *et al.* 2009). However, they reported higher levels of methanol soluble phenolics (ranging from 33 to 680 mg/100 g of grain flour) compared to our study (Lopez- Martinez *et al.* 2009). Similarly, a consistent pattern where pigmented maize varieties harbored higher concentrations of methanol soluble phenolics compared to non-pigmented counterparts was observed. The methanol soluble phenolic content in raw corn was 35 to 50 mg/100 g sample, a range similar to the lower end of methanol soluble phenolics observed in present investigation (Liu 2007).

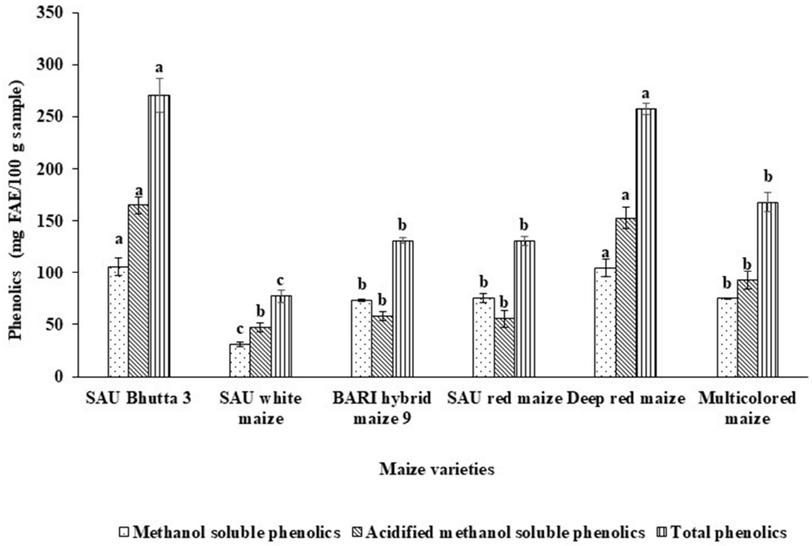

Fig. 2. Methanol soluble, acidified methanol soluble and total phenolics (mg FAE/100 g dry weight basis) in different pigmented maize varieties.

The levels of acidified methanol-soluble phenolics ranged from 47.00 ± 4.4 mg to 165.10 ± 8.0 mg FAE/100 g sample. SAU Bhutta 3 and deep red maize exhibited the highest acidified methanol soluble phenolic, measuring 165.1 ± 8.0 mg and 153.00 ± 10.0 mg FAE/100 g sample, respectively. Conversely, BARI hybrid maize 9, SAU red maize, and SAU white maize varieties showed similar levels of acidified methanol soluble phenolics (Fig. 2). Notably, SAU white maize kernels contained the lowest amount of acidified methanol soluble phenolics among the samples. Additionally, in BARI hybrid maize 9 and SAU red maize varieties, the content of acidified methanol soluble phenolics was less compared to methanol soluble phenolics, even though this content was superior in other maize varieties (Fig. 2). The maize samples displayed a higher content of acidified methanol soluble phenolics, ranging from 206 to 270 mg/100 g of dry weight (Liu 2007, Lopez- Martinez *et al.* 2009). They noted that the raw corn with high carotenoid levels exhibited the highest phenolic content, whereas the red corn showed the lowest phenolic content, contrasting with our findings. Conversely, the content of acidified methanol soluble phenolic in yellow corn was notably less compared to that observed in the present study. Specifically, yellow corn contained 13.43 ± 1.0 µmol/g sample acidified methanol soluble phenolics (Adom and Liu 2002).

The various types of maize showed a range of total phenolic content, spanning from 77.41 ± 6.0 to 271.00 ± 16 mg/100 g sample (Fig. 2). SAU Bhutta 3 and deep red maize exhibited the highest levels, with 271.00 ± 16.00 mg FAE/100 g and 258.29 ± 5.4 mg FAE/100 g sample, respectively. No significant variations were observed among BARI hybrid maize 9, SAU red maize, and multicolored maize varieties. The SAU white maize possessed the lowest total phenolic content among the samples analyzed. In a separate study, much greater total phenolic content was detected in some maize varieties than in the present study (Zilic *et al.* 2012).

The total flavonoid levels varied significantly among the maize varieties examined in the present study. The range of total flavonoid content across the maize varieties was from 9.0 ± 2.0 to 77.00 ± 10.0 mg CE/100 g sample (Table 2). The SAU Bhutta 3 exhibited the highest flavonoid concentration at 77.00 ± 10.0 mg CE/100 g sample, trailed by deep red maize at 52.0 ± 8.0 mg CE/100 g sample. Multicolored maize displayed a flavonoid content of 18.22 ± 3.0 mg CE/100 g. SAU red maize, BARI hybrid maize 9, and SAU white maize showed similar flavonoid levels while white maize demonstrated the lowest flavonoid content. Consistent with another study, multicolored maize followed by white maize exhibited the lowest flavonoid concentrations, while light and dark blue maize kernels possessed the highest. Additionally, lemon yellow, yellow, and orange maize kernels were observed to contain higher flavonoid levels compared to red and dark red maize kernels (Zilic *et al.* 2012). The flavonoid levels could differ based on the darkness of the maize kernels.

The total anthocyanin contents of the different maize varieties range were found from 4.11 ± 1.2 mg to 69.00 ± 5.3 mg C3G/100 g maize (Table 2). The SAU Bhutta 3 contained 69.00 ± 5.3 mg of C3G/100 g anthocyanin which was the highest among the maize types examined. Following that the deep red maize contained 50.00 ± 6.4 mg of C3G per 100 g sample. In contrast, the other maize varieties studied showed minimal anthocyanin content compared to the SAU Bhutta 3. Multicolored maize kernels contained approximately four times less anthocyanin than the SAU Bhutta 3. Notably, there were no significant differences observed among the anthocyanin levels in SAU white, yellow, and SAU red maize varieties. Additionally, anthocyanins were not detectable in white, yellow, lemon yellow, or orange maize kernels in one of the studies (Zilic *et al.* 2012). The highest anthocyanin content was reported in maize with dark colored pericarp such as dark red, dark blue and even in light blue and multicolored maize (Zilic *et al.* 2012). According to the reports, the quantity of anthocyanins determines the color of the pericarp. Likewise, in the present study, we noticed higher anthocyanin levels in dark-colored maize types and lower levels in the non-pigmented maize varieties. Consistent with earlier findings (Lopez-Martinez *et al.* 2009), it was also noted low anthocyanin content in SAU white maize, contrasting with high levels found in pigmented maize varieties such as SAU purple (SAU Bhutta 3) and deep red maize.

Table 3 displayed the proportions of methanol soluble and acidified methanol soluble phenolics relative to total phenolics, the proportion of flavonoids relative to insoluble phenolics, and the proportion of anthocyanins relative to total flavonoids. The proportion of methanol soluble phenolics relative to the total phenolic content varied from 39.1 to 56.00% across maize varieties, while the proportion of acidified methanol soluble phenolics ranged from 42.4 to 61.0%. With the exception of BARI hybrid maize 9 and SAU red maize varieties, the contribution of acidified methanol soluble phenolics to total phenolics exceeded that of methanol soluble phenolics. Notably, the SAU Bhutta 3 exhibited the lowest percentage contribution of methanol soluble phenolics to total phenolics, despite having high methanol soluble phenolic content. Interestingly, the percentage contributions of acidified methanol soluble phenolics to total phenolic content in SAU white maize were akin to those in SAU Bhutta 3, yet the acidified methanol soluble phenolic content in SAU white maize was least among them. Regarding flavonoids, their contribution to acidified methanol soluble phenolics ranged from 19.0 to 46.34%. SAU Bhutta 3 showed the highest proportion of flavonoids relative to acidified methanol soluble phenolics, while SAU white maize exhibited the lowest. This pattern was consistent with the total flavonoid content across the samples. Furthermore, the proportion of anthocyanins to flavonoids ranged from 28.0 to 96.0%. The deep red maize ranked the first whereas the SAU red maize exhibited the lowest, despite SAU Bhutta 3 having the highest anthocyanin content and BARI hybrid maize 9 having the lowest. The proportion of free phenolics to the total phenolic content ranged from 18 to 23%, whereas bound phenolics constituted 77 to 82% (Lopez- Martinez *et al.* 2009). In corn, bound phenolics accounted for 85% of the total phenolic content, with free phenolics comprising 15% (Adom and Liu 2002). Previous studies also indicated that bound flavonoids contributed 91% to insoluble phenolics in corn. Results of the present study, regarding methanol soluble and acidified methanol soluble phenolics align with prior findings. However, the proportion of total phenolics to acidified methanol soluble phenolics in the present study was lower than what has been previously reported.

Table 3. The percentage contributions of the free and bound fractions of maize to total phenolics, total flavonoids, and total anthocyanins.

Maize Sample	Phenolic content (%)		Flavonoid content (%)	Anthocyanin content (%)
	soluble	insoluble		
SAU white maize	39.47	60.53	18.83	64.63
BARI hybrid maize 9	55.82	44.18	21.39	37.90
SAU red maize	57.62	42.38	26.08	28.33
SAU purple maize	39.07	60.94	46.34	89.66
Deep red maize	40.72	59.28	34.03	96.24
Multicolored maize	44.67	55.32	19.61	82.88

Table 4. Correlation coefficients between functional constituents.

	TP	MSP	AMSP	TF	A
TP	1				
MSP	0.933	1			
AMSP	0.981	0.849	1		
TF	0.936	0.805	0.956	1	
A	0.940	0.780	0.975	0.992	1

Pearson's correlation analysis was conducted using averaged values of each variable (p = 0.05). TP-total phenolics; MSP-methanol soluble phenolics; AMSP-acidic methanol soluble phenolics; TF-total flavonoids; A-anthocyanins.

The correlation matrix for all the components analyzed was highly positive (Table 4). The correlation between soluble phenolics and anthocyanin content was lower than the correlation between acidic methanol-soluble phenolics and anthocyanin content. Similarly, in the present study, there was a highly positive correlation (r = 0.99) between the total phenolic and total flavonoid contents in the free, esterified and insoluble bound phenolic fractions obtained from herbs used in traditional Chinese medicine (Yu *et al.* 2019). This indicates that the total flavonoids were the major contributors to the total phenolics. However, a moderately positive correlation (r = 0.619, $p < 0.05$) between the content of total phenolics and flavonoids in ear sections of sweet corn was observed (Yang *et al.* 2019). A negative correlation between free and bound phenolics and between soluble conjugated and bound phenolics in maize was observed (Hadinezhad *et al.* 2023). However, similar to the present study, a small significant positive correlation between free and conjugated phenolics indicated that an increase in bound phenolics resulted in a decrease in both free and conjugated phenolics (Hadinezhad *et al*. 2023). A significant positive correlation between total flavonoid content and total phenol content (r = 0.730, p = 0.01) and total anthocyanin content (r = 0.343, *p* = 0.05) in maize was also established (Ku *et al.* 2014). However, they reported a non-significant correlation (r = 0.241) between total polyphenol content and total anthocyanin content in pigmented maize.

Overall, among the maize samples analyzed in the present study, the newly released SAU purple maize (SAU Bhutta 3) was superior in terms of methanol-soluble and acidified methanol-soluble polyphenols, flavonoids and anthocyanins. Furthermore, it was noted that the increase in anthocyanin levels correlated with the concentration of phenolics. SAU purple maize (SAU Bhutta 3), identified as the variety with the highest phenolic content, demonstrated the highest levels of phenolics, flavonoids, and anthocyanins. Deep red maize and multicolored maize kernels also exhibited elevated phytochemical levels. Therefore, these varieties warrant further investigation into their other phytonutrient compositions and potential health advantages.

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